

CLAIMS:

1. A bidentate motif capable of binding a cytoplasmic protein and activating
5 cellular activities in a cell, said bidentate motif comprising a tyrosine and a serine/threonine residue which are capable of interaction with cytoplasmic proteins, and wherein the residue and cytoplasmic protein can interact to activate cellular activity in the cell.

10 2. A bidentate motif according to claim 1 wherein the tyrosine and serine/threonine residue comprises a binary switch for independent regulation of cellular activity.

15 3. A bidentate motif capable of binding to a cytoplasmic protein comprising a tyrosine and a serine/threonine residue, said motif consisting of the following amino acid sequence alignment:



wherein X is any residue, Y is tyrosine, S/T is serine or threonine and Ψ is a hydrophobic residue or an equivalent thereof.

20 4. A bidentate motif capable of binding to a cytoplasmic protein comprising a tyrosine and a serine/threonine residue, said motif consisting of the following amino acid sequence alignment:



25 wherein X is any residue, Y is tyrosine, S/T is serine or threonine and Ψ is a hydrophobic residue or an equivalent thereof.

30 5. A bidentate motif capable of binding to a cytoplasmic protein comprising a tyrosine and a serine/threonine residue, said motif consisting of the following sequence alignment:



wherein X is any residue, Y is phosphotyrosine, S/T is phosphoserine/phosphothreonine.

6. A bidentate motif of a receptor molecule capable of binding to a cytoplasmic protein comprising a tyrosine and a serine/threonine residue, said motif consisting of the following amino acid sequence alignment:



5 wherein X is any residue, Y is tyrosine, S/T is serine or threonine and Ψ is a hydrophobic residue or an equivalent thereof.

7. A bidentate motif of a receptor molecule capable of binding to a cytoplasmic protein comprising a tyrosine and a serine/threonine residue, said
10 motif consisting of the following amino acid sequence alignment:



wherein X is any residue, Y is tyrosine, S/T is serine or threonine and Ψ is a hydrophobic residue or an equivalent thereof.

15 8. A bidentate motif of a receptor capable of binding to a cytoplasmic protein comprising a tyrosine and a serine/threonine residue, said motif consisting of the following sequence alignment:



20 wherein X is any residue, Y is phosphotyrosine, S/T is phosphoserine/phosphothreonine.

9. A bidentate motif according to any one of claims 1 to 8 from a receptor selected from the group including

- (1) GM-CSF/IL-3/IL-5 receptor
- 25 (2) IL6 human interleukin-6 receptor beta chain precursor (IL-6R-beta)
- (3) LEPR human leptin receptor precursor (LEP-R) (OB RECEPTOR) (OB-R).
- (4) TNR2 human tumor necrosis factor receptor 2 precursor (tumor necrosis factor
- 30 (5) VGR1 human vascular endothelial growth factor receptor 1 precursor
- (6) TRK3 human receptor protein-tyrosine kinase TKT precursor (EC 2.7.1.112)
- (7) Q01974 protein-tyrosine kinase transmembrane receptor ROR2 precursor

- (8) FGR1 human basic fibroblast growth factor receptor 1 precursor (BFGF-R)
- (9) Q15426 protein-tyrosine phosphatase, receptor-type, H precursor (EC 5 3.1.3.48)
- (10) PTPM human protein-tyrosine phosphatase mu precursor (EC 3.1.3.48) (R-PTP-MU).
- (11) PDGS human alpha platelet-derived growth factor receptor precursor (EC 2.7.1.112)
- 10 (12) FGR4 human fibroblast growth factor receptor 4 precursor (FGFR-4) (EC 2.7.1.112)
- (13) FGR2 human fibroblast growth factor receptor 2 precursor (FGFR-2) (EC 2.7.1.112)
- (14) Q13635 patched protein homolog (PTC)
- 15 (15) MANR human macrophage mannose receptor precursor.
- (16) LRP2 human low-density lipoprotein receptor-related protein 2 precursor (megalin)
- (17) IDD human integral membrane protein dgcr2/idd precursor (KIAA0163)
- (18) AMFR human autocrine motility factor receptor precursor (AMF receptor)
- 20 (gp78)
- (19) ACH5 human neuronal acetylcholine receptor protein, alpha-5 chain precursor.
- (20) KK1T human: stem cell growth factor receptor (proto-oncogene tyrosine-protein kinase kit) (C-KIT) (CD117 antigen)
- 25 (21) TPOR human: thrombopoietin receptor precursor (TPO-R) (myeloproliferative leukemia protein (C-MPL). TPOR or MPL.
- (22) TPOR mouse: thrombopoietin receptor precursor (TPO-R) (myeloproliferative leukemia protein) (C-MPL). TPOR or MPL.
- (23) Acetylcholine R
- 30 (24) Acetylcholine R alpha-5
- (25) C-C chemokine receptor 6
- (26) Middle T antigen
- (27) integrin alpha 1

- (28) FGFR2 (KGF R)
- (29) FGFR1 (flg)
- (30) FGFR5
- 5 (30) Erb4
- (31) Vaccinia virus protein A36R
- (32) Macrophage mannose R (MRC1)
- (33) LDLR
- (34) VLDL (rat)
- 10 (35) LRP1 low density lipoprotein receptor-related protein 1
- (36) integrin beta 1
- (37) interin beta 7
- (38) integrin beta 3
- (39) integrin beta 5
- 15 (40) integrin beta 6
- (41) G-CSFR1 (second)
- (42) g-csf-r
- (43) IL-6B (gp130)
- (44) LeptinR
- 20 (45) ProlactinR
- (46) insulinR
- (47) irs-1
- (48) IGFI R
- (49) flt3 R
- 25 (50) VEGFR2 (FLK1)
- (51) PDGF R-alpha
- (52) IL-9R
- (53) Beta R
- (54) Neuronal acetylcholine receptor protein, alpha-3 chain
- 30 (55) protein tyrosine phosphatase receptor N
- (56) glycogen synthase kinase 3 alpha
- (57) p21-activated kinase 3
- (58) 3-phosphoinositide dependent protein kinaes-1 (PDK1)
- (59) integrin alpha 1 (laminin/collagen receptor)

or a functional equivalent or analogue thereof.

10. A bidentate motif according to any one of claims 1 to 9 having a sequence selected from the group including:

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NGPYLG.....PP..HSRSLP
NVHYRT.....P...KTHTMP
**RYFTQKEE.....TESGSGP
NKKYELQDRDVCE....P.RYRSVSEP

10

NPTYSVM.....RSHSYP
NIFYLIR...KSGSFPMPELKLSISFP
NEEYLDLSQ.....PLEQYSPSYP
NQEYLDLSM.....PLDQYSPSFP
NATYKVD.....VIQRTRSKP

15

NPEY.....HSASSGP
NPDY.....WNHSLP
NPSYSSNPFVNYN....KTSICSKSNP
NTLY.....FNSQSSP
NPVYQKTTEDEVHI...CHNQDGYSYP

20

NPVYLKTTEEDLSIDIG..RH.SASVG
NPTYKMYEGGEPPDDVGGLLDADFALDPDKPTNFTNPVY
NPIY.....KSAVTTVV

NPLY.....KSAITTTV
NPLY.....KEATSTFT

25

NPLY.....RKPISTHT
NPLY.....RGSTSTFK
PGHYL.....RCDSTQP
VQTYVLQ.....GDPRAVSTQP
QVLYGQLL.....GSPTSP

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HSGYRHQVPSVQVF....SRSESTQP
WKM**YEVYDA**.....Ks.KSVSLP
KIPYFHA.....GGS.KCSTWP
ELDYCLKGLKL.....P.S.RTWSPP
SGDYMPM.....SPKSVSAP

35

SFYYSEENKLPEPEELDLEPENMESVP(LDPSASSSSLP)
EEIYIIM....QSCWAFDSRKRPSFP
ISQYLQN.....S.KRKSRP
GTAY.....GLSRSQP
***YLPQEDWAP.....TSLTRP

40

LVAYIAFKRWNSCKQN...KQGANRPVNQT~~PP~~GEKLHSDSGIS

11. A bidentate motif according to any one of claims 1 to 10 wherein the motif is derived from a cytokine receptor.

45 12. A bidentate motif according to any one of claims 1 to 11 wherein the cytokine receptor is the GM-CSF/IL-3/IL-5 receptor.

13. A bidentate motif according to any one of claims 1 to 12 wherein the motif is derived from the common beta chain (βc).
14. A bidentate motif according to any one of claims 1 to 13 wherein the Tyr 5 is equivalent to Tyr577 of the common beta chain (βc).
15. A bidentate motif according to any one of claims 1 to 14 wherein the Ser is equivalent to Ser 585 of the common beta chain (βc).
- 10 16. A bidentate motif according to any one of claims 1 to 15 wherein the tyrosine or serine/threonine independently phosphorylate in response to cytokine concentration
- 15 17. A bidentate motif according to any one of claims 1 to 16 wherein phosphorylation of the serine independently of the tyrosine regulates cell survival.
18. A bidentate motif according to claim 16 or 17 wherein the cytokine concentration is less than 10pM, preferably 3pM, more preferably 1pM.
- 20 19. A bidentate motif according to any one of claims 1 to 16 wherein phosphorylation of the tyrosine independently of the serine regulates cell survival and proliferation.
- 25 20. A bidentate motif according to claim 18 or 19 wherein the cytokine concentration is greater than 10pM.
- 30 21. A bidentate motif according to any one of claims 1 to 20 which binds to at least one cytoplasmic protein selected from the group including 14-3-3 protein, Shc, SHIP-2, WW-domain of the prolyl isomerase, Pin1 and the ubiquitin ligase, NEDD4.
22. A bidentate motif according to claim 21 wherein the cytoplasmic protein is 14-3-3, Shc or SHIP-2.

23. A bidentate motif according to any one of claims 1 to 22 wherein the Tyr binds to the Shc.

5 24. A bidentate motif according to any one of claims 1 to 23 wherein the Ser binds to 14-3-3.

25. A bidentate motif according to any one of claims 1 to 20 having a modification at a residue equivalent to the Tyr and/Ser.

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26. A bidentate motif according to claim 25 wherein the residue equivalent to Tyr is substituted with Phe.

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27. A bidentate motif according to claim 25 or 26 wherein the Ser residue is substituted with Gly.

28. A method of modulating cellular activity in a cell, said method comprising: modifying phosphorylation of a Tyr and/or Ser residue of a bidentate motif according to any one of claims 1 to 27.

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29. A method according to claim 28 wherein the Tyr is equivalent to Tyr577 and Ser is equivalent to 585 of the common beta chain (β c) in a cell.

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30. A method according to claim 29 wherein the common beta chain (β c) is from the GM-CSF/IL-3/IL-5 receptor.

31. A method according to any one of claims 28 to 30 wherein the cellular activity is modulated by increasing or decreasing phosphorylation of the Tyr and/or Ser residue of the bidentate motif.

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32. A method according to claim 31 wherein the phosphorylation is increased by subjecting the cell to a phosphorylating agent.

33. A method according to claim 32 wherein the phosphorylating agent is a kinase.

5 34. A method according to claim 31 wherein the phosphorylation is decreased by mutating the Tyr and/or Ser.

35. A method according to claim 34 wherein the Tyr is substituted for Phe and/or the Ser is substituted for Gly.

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36. A method according to claim 31 wherein the phosphorylation is decreased by subjecting the cell to an antagonist which inhibits phosphorylation of the Tyr and/or Ser.

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37. A method according to claim 31 wherein the phosphorylation is decreased by subjecting the cell to a kinase inhibitor to inhibit phosphorylation of the Tyr and/or Ser.

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38. A method according to claim 31 wherein the phosphorylation is modulated by exposing the cell to a cytokine.

39. A method according to claim 38 wherein the cytokine is GM-CSF.

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40. A method according to any one of claims 28 to 38 for inhibiting cellular activity, said method comprising decreasing or inhibiting phosphorylation of the Tyr and/or Ser of the bidentate motif.

41. A method according to claim 40 wherein the cellular activity is cell survival, said method comprising inhibiting phosphorylation of the serine.

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42. A method according to claim 41 wherein the serine is equivalent to Ser585 of the common beta chain (βc).

43. A method according to any one of claims 40 to 42 for inhibiting cellular activity, said method further comprising inhibiting binding of a cytoplasmic protein to the bidentate motif.

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44. A method according to claim 43 wherein the cytoplasmic protein is selected from the group including 14-3-3 protein, Shc, SHIP-2, WW-domain of the prolyl isomerase, Pin1 and the ubiquitin ligase, NEDD4.

10 45. A method according to claim 43 or 44 wherein the cytoplasmic protein is 14-3-3 or Shc.

46. A method according to any one of claims 28 to 38 for activating cellular activity, said method comprising inducing phosphorylation of the Tyr and/or Ser 15 of the bidentate motif.

47. A method according to claim 46 wherein the cellular activity is cell survival, said method comprising increasing phosphorylation of the serine.

20 48. A method according to claim 47 wherein the cell is exposed to GM-CSF at a concentration of up to 10pM, preferably 3pM, more preferably 1pM.

49. A method according to claim 46 wherein the cellular activity is cell proliferation, said method comprising increasing phosphorylation of the tyrosine.

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50. A method according to claim 49 wherein the cell is exposed to at least 10pM. GM-CSF.

30 51. A method according to any one of claims 28 to 50 further including modifying an interaction between the cytoplasmic protein 14-3-3, Shc and Ty179 on the 14-3-3.

52. A method according to claim 51 including inhibiting an interaction between the cytoplasmic protein 14-3-3, Shc and Tyr 179 on the 14-3-3.

53. A method according to any one of claims 28 to 52 wherein the cell is a haematopoietic cell.
54. A method of treating a cytokine mediated condition, said method comprising:
5 regulating activation of phosphorylation the tyrosine and/or serine of a bidentate motif according to any one of claims 1 to 27.
- 10 55. A method according to claim 54 wherein the Tyr is equivalent to Tyr 577 and Ser is equivalent to Ser 585 of the common beta chain (βc).
56. A method according to claim 54 or 55 wherein the common beta chain (βc) is of the GM-CSF/IL-3/IL-5 receptor.
- 15 57. A method according to any one of claims 54 to 56 wherein the cytokine mediated condition is treated by increasing or decreasing activation of phosphorylation of the tyrosine and/or serine of the bidentate motif.
- 20 58. A method according to claim 57 wherein the phosphorylation is increased by subjecting the cell to a phosphorylating agent.
59. A method according to claim 58 wherein the phosphorylating agent is a kinase.
- 25 60. A method according to claim 57 wherein the phosphorylation is decreased by mutating the Tyr and/or Ser.
61. A method according to claim 60 wherein the Tyr is substituted for Phe and/or the Ser is substituted for Gly.
- 30 62. A method according to claim 57 wherein the phosphorylation is decreased by subjecting the cell to an antagonist which inhibits phosphorylation of the Tyr and/or Ser.

63. A method according to claim 57 wherein the phosphorylation is decreased by subjecting the cell to a kinase inhibitor to inhibit phosphorylation of the Tyr and/or Ser.
- 5 64. A method according to claim 54 wherein the phosphorylation is regulated by exposing the cell to a cytokine.
65. A method according to claim 64 wherein the cytokine is GM-CSF.
- 10 66. A method according to any one of claims 54 to 65 wherein the cytokine mediated condition is a GM-CSF mediated condition.
67. A method according to claim 54 wherein the cytokine mediated condition involves cell survival.
- 15 68. A method according to claim 67 for improving cell survival, said method including subjecting the cell to GM-CSF.
69. A method according to claim 68 wherein the GM-CSF is at a
- 20 concentration of up to 10pM, preferably 3pM, more preferably 1pM.
70. A method according to claim 54 wherein the cell modulated condition involves cell proliferation.
- 25 71. A method according to claim 70 for improving cell proliferation, said method comprising subjecting the cell to GM-CSF.
72. A method according to claim 71 comprising subjecting the cell to greater than 10pM GM-CSF.
- 30 73. A method according to claim 54 wherein the cytokine indicated condition is carrier.

74. A method according to claim 73 comprising inhibiting phosphorylation of the tyrosine and serine.

75 .A method according to claim 54 wherein the cytokine mediated condition 5 is selected from the group including myeloid cell activation, asthma and rheumatoid arthritis.

76. A method for diagnosing a proliferative condition involving cell proliferation or cell survival, said method including:

10 detecting a level of phosphorylation of Tyr and/or Ser in a bidentate motif according to any one of claims 1 to 27 in a cell; and
comparing against a cell of a normal level of phosphorylation.

77. A method according to claim 76 wherein the Tyr is equivalent to Tyr577 15 and Ser is equivalent to Ser585 of the common beta chain (β c).

78. A method according to claim 76 or 77 wherein the common beta chain (β c) is from the GM-CSF/IL-3/IL-5 receptor.

20 79. A method according to any one of claims 76 to 78 wherein the cell is a haematopoietic cell.